IN THE UNITED STATES PATENT AND TRADE MARK OFFICE

In re Application of)
STEPHEN P. COBBOLD *et al*)
Serial No. 08/289,532)
Filed 12th August 1994)
For: MONOCLONAL ANTIBODIES)
FOR INDUCING TOLERANCE)

Examiner: Gambel

Group Art Unit: 1806

DECLARATION

I, JEFFREY MONROE JOHNSTON of Glaxo Wellcome Inc., Research Triangle Park, North Carolina do hereby solemnly and sincerely declare as follows:

1. INTRODUCTION

1.1 I received the degree of B.Sc. Summa Cum Laude, from Davidson College, Davidson, North Carolina in 1974 and the degree of M.D. from Duke University School of Medicine, Durham, North Carolina in 1977. During 1978 I was Fellow in Rheumatic and Genetic Diseases at Duke University School of Medicine and from 1978 to 1980 I was Intern and Resident in Medicine at Vanderbilt University Affiliated Hospitals, Nashville, Tennessee. From 1980 to 1982 I was a Senior Assistant Surgeon at the Centers for Disease Control (E.I.S.) of the U.S Public Health Service. From 1982 to 1983 I was Chief Medical Resident at the University of Utah School of Medicine, Salt Lake City, Utah and from 1983 to 1985 Fellow in Infectious Diseases at the same Medical School.

1.2 FROM 1985 to 1987 I was Assistant Professor in the Department of Internal Medicine at the University of Utah School of Medicine and from 1987 to 1988 I was Assistant Professor in the Departments of Medicine and Microbiology at the University of Texas Health Science Center, San Antonio, Texas.

1.3 FROM 1988 to 1995 I was employed by Burroughs Wellcome Co., Research Triangle Park, North Carolina and from 1995 to date I have been employed by Glaxo Wellcome Inc., Research

Triangle Park, North Carolina. From 1988 to 1995 I was Head of the Clinical Immunology Section of the Medical Affairs Division of Burroughs Wellcome Co. From 1992 to 1994 I was also Associate Director of the Department of Infectious Disease and Immunology and from 1994 to 1995 Associate Director of the Clinical Oncology Group of the Medical Affairs Division of Burroughs Wellcome Co. I am currently Senior Clinical Program Head of the Immunology Program within International Anti-Infectives and Immunology Clinical Research at Glaxo Wellcome Inc.

1.4 FROM 1988 to 1994, I also held the position of Clinical Associate Professor in the Department of Medicine at the University of North Carolina School of Medicine, Chapel Hill, North Carolina and from 1994 to date I have held the position of Clinical Professor in the same Department. I am an author of well over 30 scientific papers in the field of medicine published from 1978 to date.

1.5 I have been involved from its inception in 1992 in the project, first at Burroughs Wellcome and then at Glaxo Wellcome, to develop an anti-CD4 antibody for use in autoimmune disease and I am currently International Project Leader and Medical Advisor for this project

2. CLINICAL TRIAL PROTOCOL

2.1 THIS declaration sets out a preliminary report of the results of a pilot clinical trial of a humanised IgG monoclonal antibody directed against the human CD4 molecule in patients with severe rheumatoid arthritis. Full details of the anti-CD4 antibody used in the trial are given in Gorman *et al*, Proc. Natl. Acad. Sci. (USA), *88*, 4181-4185 (1991). The trial is being carried out at Guy's Hospital, London, UK, and since the final patient was only enrolled at the end of January 1996 and the duration of the study is 3 months, the trial will not close until the end of April 1996. Accordingly, all data reported herein are preliminary and are not quality assured.

2.2 THE trial is an open-label, cohort design, dose escalation study to investigate the effect of the administration of the antibody by intravenous (iv) infusion at four escalating dose levels (10, 30, 100 and 300mg daily) in patients with active rheumatoid arthritis who have failed (due to toxicity or inadequate disease control) with at least one disease-modifying anti-rheumatic drug.

The primary objectives of this study are to evaluate safety and dose tolerance of the multi-dose schedule, to examine the pharmacodynamic and pharmacokinetic properties of the anti-CD4 antibody, to monitor disease activity, and to measure any anti-globulin or anti-idiotype response to the therapeutic antibody.

2.3 PATIENTS (six per cohort) received iv infusions of the anti-CD4 antibody as follows:

Cohort 1	10 mg daily for 5 consecutive days
Cohort 2	30 mg daily for 5 consecutive days
Cohort 3	100 mg daily for 5 consecutive days
Cohort 4	300 mg daily for 5 consecutive days.

All patients are being evaluated for acute toxicity, pharmacokinetics, pharmacodynamics, lymphocyte studies and therapeutic response for a period of three months.

3. PRELIMINARY CLINICAL RESULTS

3.1 CLINICAL activity assessments, including swollen joint counts (n = 28) were carried out at screen (no more than 4 weeks prior to the start of treatment), baseline (immediately prior to antibody infusion) and on study days 7, 14, 28, 42, 56 and 84 (end of study). The results have been presented as a graph a copy of which I now provide as Exhibit "JMJ1". The patients enrolled into the study had very active disease with the median joint count ranging from 24 to 27 at screen and 19 to 28 at baseline among the four treatment groups. Despite the limitation imposed by the small size of the treatment groups (n = 6), there was a clear dose-related improvement in joint swelling as manifest by reductions in the number of swollen joints during the study. Very little change was noted in the lowest dose group (10mg iv daily for 5 days). There was a modest but steady fall in the value for median swollen joints in the 30mg cohort over the duration of the study (25% improvement by the end of the 12 week study period). Dramatic improvement occurred in the 100mg and 300mg patients with rapid falls in the median joint count by the end of the first week and reaching 58% and 67% improvement from baseline, respectively, four weeks from the start of dosing. It should be noted that although the final point on the graph for the 300mg group appears to show an increase in the median joint count relative to the preceding point, at the time

that the data was collected for this graph only two of the patients in the final (300mg) cohort had reached the day 84 assessment. Accordingly, this final point on the graph is not representative of the whole group in the sense that only 2 out of the 6 patients in the group are included.

3.2 WESTERGREN Erythrocyte Sedimentation Rate (ESR) was also measured at the same study visits as the swollen joint counts. The results have again been presented as a graph a copy of which I now provide as Exhibit "JMJ2". As with the swollen joint counts, the graph appears to show a reasonable dose response in the change in ESR following infusion of the anti-CD4 antibody. With some variation, the two lower dose cohorts showed steady increases (worsening) in the median ESR, whereas the two higher dose cohorts demonstrated improvement. The median ESR in both the 100mg and 300mg cohorts improved during the first week reaching levels roughly 40% below baseline values. These reductions in acute phase reactant were sustained through approximately 6 weeks from the start of treatment and then began to return towards baseline. Again, as with swollen joint counts, the data from the high dose cohort is incomplete and the figure for the 84 day assessment is not based on data for the whole cohort.

4. BIOLOGICAL ACTIVITY OF THE ANTI-CD4 ANTIBODY

4.1 ONE effect of treatment with the anti-CD4 antibody was the loss (down-modulation) of CD4 molecules from the T-lymphocyte surface membrane. This effect was determined by FACS analysis using a non-competing anti-CD4 antibody labelled with fluorescein (Leu 3a, Becton Dickinson) and comparing the intensity of staining before and at intervals after treatment. The results have been presented as a graph a copy of which I now provide as Exhibit "JMJ3". Rapid down modulation of CD4 was seen after all doses of the anti-CD4 antibody, with the maximum extent of the effect varying between 80 and 99%. Both the degree of down-modulation and its duration were related to the dose of the anti-CD4 antibody administered. Synovial fluid lymphocytes were obtained from all of the 100mg cohort patients and 2 of the 300mg cohort patients at varying time points and down-modulation of CD4 was seen up to 2 weeks and up to 4 weeks from the start of treatment in the 100mg and 300mg cohorts respectively.

4.2 T-CELL proliferative responses were measured by separating blood lymphocytes and culturing them in the presence of OKT3, which is a T-cell specific mitogen. Responses were

measured by uptake of tritiated thymidine and recorded as stimulation index. Although not complete, sufficient data are currently available for the 30mg and 100mg cohorts to be representative of the groups and these data have been presented as a graph a copy of which I now provide as Exhibit "JMJ4". Significantly less data are available as yet for the 300mg cohort and this has not been included.

5. ANTIBODY RESPONSE TO THE ANTI-CD4 ANTIBODY

5.1 PLASMA was collected from all patients before and at intervals after treatment up to day 84. A sensitive sandwich ELISA technique capable of detecting antibodies against either common or idiotypic determinants on the 4162W84 molecule was used to test these samples and no such antibodies were detected.

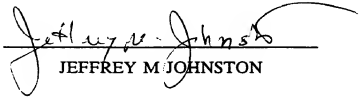
6. CONCLUSIONS

6.1 THE preliminary results of this pilot clinical study in patients with severe rheumatoid arthritis may be interpreted tentatively (given the small number of patients) as follows:

- Treatment with the anti-CD4 antibody resulted in profound, dose-related, down-modulation (80 to 99%) of CD4 molecules from the surface of both circulating and synovial T-lymphocytes, showing that the antibody exerts a significant biological effect *in vivo*, including at the site of the disease.
- There was a dose-related improvement in both clinical and biological measures of disease activity, exemplified by swollen joint count and ESR.
- None of the patients developed antibodies against the humanised therapeutic antibody following multiple infusions, suggesting that it may prevent an antibody response to itself.
- This preliminary evidence of *in vivo* biological activity, disease improvement, and lack of anti-idiotype response support further dosing studies of the anti-CD4 antibody in patients with rheumatoid arthritis, aimed at inducing a state of immunological tolerance among their $CD4^+$ T-lymphocytes, resulting in prolonged clinical benefit.

7. DECLARATION

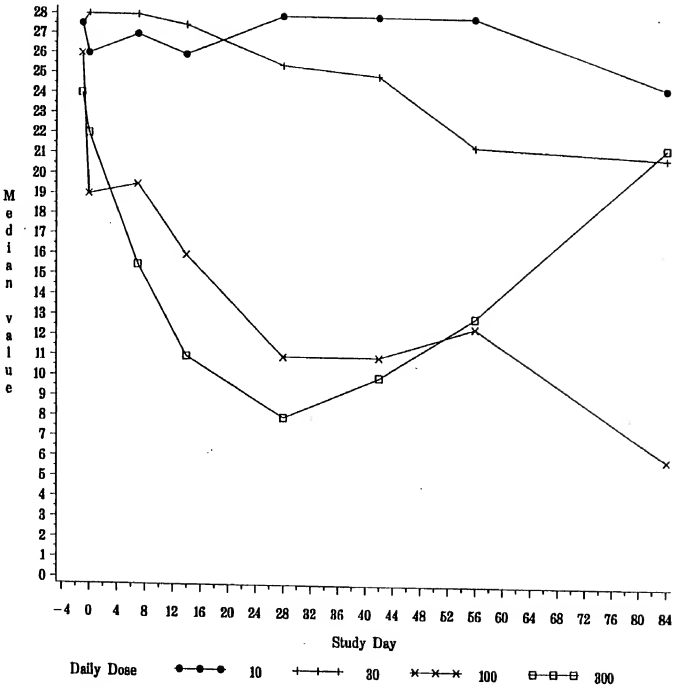
7.1 I further declare that all statements made herein to my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.



JEFFREY M JOHNSTON

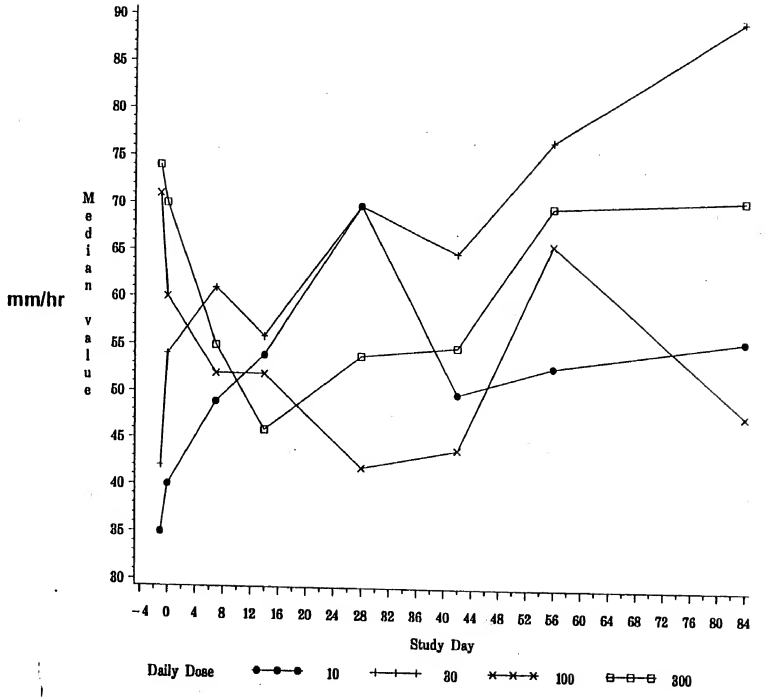
Date: 27 March '96

Joint Swelling



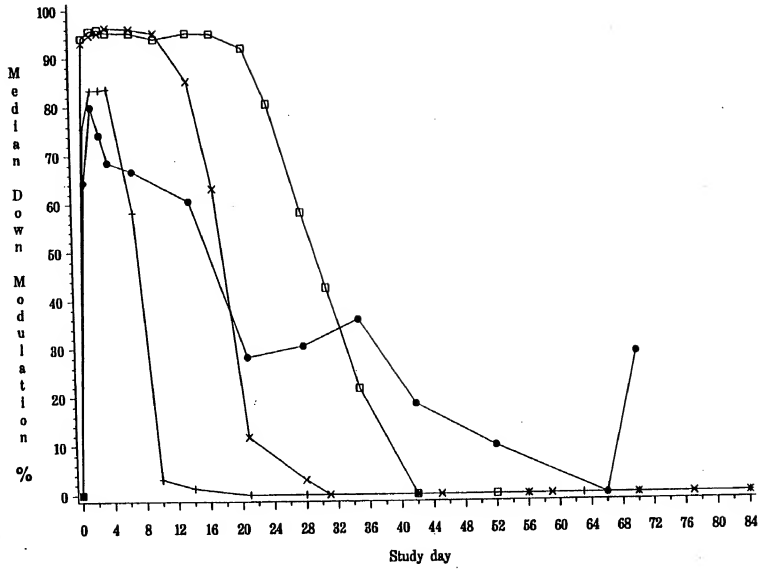
JMJ 1

ESR


mm/hr
Median value
90
85
80
75
70
65
60
55
50
45
40
35
30
-4 0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84
Study Day
Daily Dose
10
30
100
300

JMJ 2

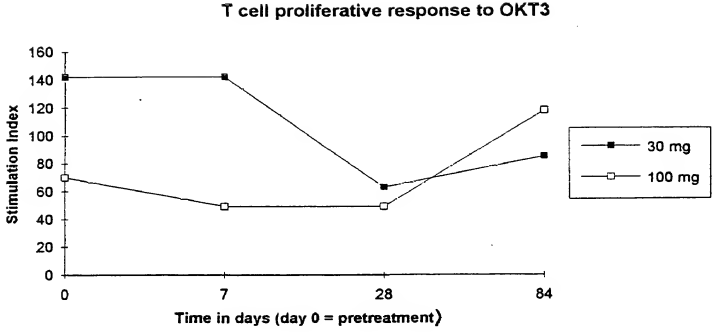
Down-Modulation of CD4



JMJ 3

Post-dose values for days 0 and 4 are excluded

JMJ 4


T cell proliferative response to OKT3
Stimulation Index
160
140
120
100
80
60
40
20
0
0
7
28
84
Time in days (day 0 = pretreatment)
30 mg
100 mg